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Prefrontal cortical abnormalities in currently depressed versus currently remitted patients with major depressive disorder

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Abstract

Previous neuromorphometric investigations of major depressive disorder (MDD) have reported abnormalities in gray matter in several regions, although the results have been inconsistent across studies. Some discrepancies in the results across studies may reflect design limitations such as small sample sizes, whereas others may reflect biological variability that potentially manifests as differences in clinical course. For example, it remains unclear whether the abnormalities found in persistently depressed MDD subjects extend to or persist in patients who experience prolonged remission. The aim of the present study was to investigate gray matter (GM) differences in unmedicated, currently-depressed participants (dMDD) and unmedicated, currently-remitted (rMDD) participants with MDD compared to healthy controls (HC).

The GM density and volume was compared across groups using voxel-based morphometry, a quantitative neuroanatomical technique, and high-resolution MRI images from 107 HC, 58 dMDD and 27 rMDD subjects.

Relative to the HC group the dMDD group had reduced GM in the dorsal anterolateral (DALPFC), the dorsomedial (DMPFC) and the ventrolateral prefrontal cortex (VLPFC). Relative to the rMDD group the dMDD group showed reduced GM in the DALPFC, the VLPFC, the anterior cingulate cortex (ACC), the precuneus and the inferior parietal lobule. No regions were identified in which the rMDD group showed significantly lower GM compared to the HC group after p-values were corrected for the number of comparisons performed.

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In unmedicated patients in the depressed phase of MDD, we found evidence of morphometric abnormalities in DALPFC and in medial prefrontal cortical regions belonging to the visceromotor network. These findings, along with the absence of GM abnormalities in the remitted sample imply a possible link between greater GM tissue and better clinical outcome. Consistent with other neuroimaging and post-mortem neuropathological studies of MDD, we also found evidence of decreased white matter in patients with dMDD and rMDD.

Keywords

Voxel-based morphometry (VBM); remission; dorsolateral prefrontal cortex (DLPFC); gray matter; clinical outcome

1. Introduction

Voxel-based morphometry (VBM) is a fully automated method for analyzing neuromorphological MRI data that allows unbiased investigation of inherent differences in brain structure between clinical and control samples. This approach has been applied to identify neuromorphometric abnormalities in various neurodegenerative and psychiatric disorders, including major depressive disorder (MDD) (Ridgway et al., 2008; Ashburner and Friston, 2000). Structural abnormalities in MDD as reported in the VBM literature encompass regions belonging to the visceromotor network, including the orbitofrontal (OFC), subgenual prefrontal (SGPFC) and anterior cingulate cortices, and the hippocampus/amygdala complex (Tang et al., 2007; Wagner et al., 2008; Vasic et al., 2008; Leung et al., 2009; Frodl et al., 2008a; Treadway et al., 2009). However, the findings reported have been inconsistent, potentially due to experimental design differences across studies pertaining to patient selection, statistical power, or neuromorphometric methodology (Konarski et al., 2008). In addition, many published studies have used relatively small sample sizes (Tang et al., 2007; Wagner et al., 2008; Vasic et al., 2008; Leung et al., 2009; Treadway et al., 2009) or have not applied corrections for multiple testing (Tang et al., 2007; Wagner et al., 2008; Vasic et al., 2008; Leung et al., 2009). For example, decreased gray matter in the anterior cingulate cortex (ACC) has been described by some (Tang et al., 2007; Vasic et al., 2008) but not other studies (Pizzagalli et al., 2004), and the portion of the anterior cingulate affected has differed across the positive studies. Similarly, reduced amygdalar and hippocampal volume has been reported by some (Tang et al., 2007; Wagner et al., 2008), but not other studies (Frodl et al., 2008a; Treadway et al., 2009). According to recent evidence, hippocampal abnormalities might extend to treatment-naïve, first-episode MDD subjects, providing further evidence of the key role of this structure in the pathophysiology of depression (Zou et al., 2010).

It is noteworthy that in a study which employed a relatively large sample size (77 MDD patients and 77 healthy controls) and where statistical correction for multiple testing was used (Frodl et al., 2008a), structural gray matter (GM) reductions in MDD subjects were evident in cortical regions but not in subcortical structures, with the exception of the thalamus. However, most of the patients (79%) enrolled in this study were receiving antidepressant drugs at the time of scanning, potentially limiting the interpretability and generalizability of the results, particularly given previous evidence that these agents upregulate neurotrophin expression (Duman and Monteggia, 2006), contribute to neuronal remodeling (Bessa et al., 2009) and protect against gray matter loss (Lavretsky et al., 2005; Sheline et al., 2003). Therefore, whether prolonged periods of clinical remission or ongoing treatment with antidepressant medications influence gray matter volume or density remains unclear. Previous studies have shown that GM volumetric reductions associated with MDD persist in the subgenual anterior cingulate cortex (sgACC) after a mean of 4 months of

effective antidepressant drug treatment (Drevets et al., 1997), while other reports suggest that chronic antidepressant treatment might protect against further gray matter loss in brain region involved in mood-regulation, such as the orbitofrontal cortex and the hippocampus (Lavretsky et al., 2005; Sheline et al., 2003). Nevertheless, reductions in hippocampal gray matter persisted in unmedicated MDD subjects despite a prolonged period of clinical remission (Neumeister et al., 2005). Potentially highlighting the importance of clinical remission in preventing further reductions in GM volume, the results of a 3-year longitudinal study showed that depressed patients who experience stable clinical remission have less GM decline in the left hippocampus, left anterior cingulate, left dorsomedial prefrontal cortex and bilateral dorsolateral prefrontal cortex than patients who do not remit (Frodl et al., 2008b). In this study the proportion of subjects receiving antidepressant treatment did not differ between remitters and non-remitters, suggesting a potential link between greater gray matter and better clinical outcome independently from drug treatment. Notably, most of the patients participating in this study were receiving antidepressant medication at the time of first scan and a large proportion of them continued treatment throughout the follow-up period, thus limiting the interpretation of the findings. Nevertheless, taken together the results of these studies suggest that effective antidepressant drug treatment may protect against progressive GM loss without reversing extant reductions in GM volume, and raise the hypothesis that MDD patients who develop either less or no GM atrophy during depressive episodes show better outcomes during treatment.

Providing further support to the hypothesis that morphometric abnormalities might be related to illness chronicity and poor clinical outcome in depressed patients, in another VBM study Li and colleagues found that only patients who continued being symptomatic after a 6-week course of antidepressant treatment showed pre-treatment reductions in the dorsal anterolateral prefrontal cortex GM compared to healthy subjects, while these abnormalities were not apparent in subjects who experienced symptomatic remission (Li et al., 2010). However, it should be noted that those findings reflect acute response to antidepressant treatment rather than stable adaptive changes reflecting sustained clinical improvement.

The aim of the present study was to investigate GM abnormalities in unmedicated, currently-depressed patients and unmedicated patients with MDD in full remission using VBM. We hypothesized that currently depressed patients would show GM reductions relative to healthy controls and to persistently remitted MDD subjects in brain areas involved in the extended visceromotor network of structures that supports mood regulation (Drevets et al., 2008). Regional white matter (WM) differences between groups also were investigated.

2. Material and methods

2.1 Participants

Fifty-eight unmedicated patients who met DSM-IV criteria for a current major depressive episode (MDE) and either recurrent or chronic MDD (dMDD), twenty-seven remitted, unmedicated patients with a past history of at least two MDEs who currently met DSM-IV criteria for recurrent MDD in full remission (rMDD), and one-hundred-seven healthy controls (HC) participated (Table 1). Patients in remission with a history of single MDE were excluded from the study as well as dMDD patients who presented with a first episode of MDD lasting less than 2 years. Subjects provided written consent as approved by the NIH Combined Neuroscience Institutional Review Board. Participants underwent a screening evaluation prior to enrollment that involved a medical and psychiatric history, laboratory testing, drug screening, physical examination and structural magnetic resonance imaging (MRI) scanning. Psychiatric diagnoses were established by both an unstructured clinical interview conducted by a psychiatrist and the *Structured Clinical Interview for the* DSM-IV

(First et al., 2002). Subjects were excluded from participation if they had serious suicidal ideation or behavior as assessed through a clinical interview with a psychiatrist, major medical or neurological disorders, exposure to psychotropic drugs within 3 weeks (8 weeks for fluoxetine), a history of drug or alcohol abuse within 1 year or a lifetime history of drug (excepting nicotine) or alcohol dependence (DSM-IV criteria), current pregnancy or breastfeeding. Additional exclusion criteria applied to the HC subjects were having a personal history of any major psychiatric disorder or a first-degree relative with a mood or anxiety disorder. Additional exclusion criteria applied to the rMDD subjects were having experienced a depressive episode or having received psychotropic medications within the three months prior to scanning.

2.2 MRI procedure

Anatomical MRI scans were acquired on a GE 3-T Signa scanner (Milwaukee, WI) using a T1-weighted gradient echo pulse sequence (MP-RAGE) optimized for white matter/gray matter contrast (TE=2.1 ms, TR=7.8 ms, prep time=725 ms, flip angle=6, TD=1400 ms). 124 axial slices at a thickness of 1.2 mm were acquired with a field of view of 22 cm for an in-plane resolution of 0.85 × 0.85 mm. All images were visually checked for major artifacts.

2.3 VBM preprocessing

Data were processed using MATLAB 7.0 (Mathworks Inc., Natick, MA, USA) and Statistical Parametric Mapping (SPM5, Wellcome Department of Cognitive Neurology, www.fil.ion.ucl.ac.uk). Structural differences between groups were investigated using the VBM5 toolbox implemented by the Structural Brain Mapping Group of the University of Jena (http://dbm.neuro.uni-jena.de/vbm). The VBM5 toolbox extends the unified segmentation approach implemented in SPM5, which integrates tissue classification, spatial normalization and intensity inhomogeneity bias correction (Ashburner and Friston, 2005). This algorithm affords significant advantages over the optimized VBM procedure by improving segmentation accuracy, obviating the need to create a study-specific template prior to segmentation, and avoiding the “circularity problem” inherent in optimized VBM (Ashburner and Friston, 2005). We used the iterative Hidden Markov Random Field option which introduces spatial constraints based on neighboring voxels intensities within a 3×3×3 voxel cube in order to remove isolated voxels which are unlikely to belong to a determinate tissue class. The International Consortium Brain Mapping (ICBM) tissue priors were not used for the tissue classification in order to improve the segmentation accuracy in subjects who deviate from the ICBM tissue distribution. Spatial normalization was performed using both affine and non-linear registration, with warping regularization=1. Both volume and density gray matter images were assessed. Modulated “volume” images were multiplied by the determinant of the spatial transformation matrix, so that the original gray matter volume (GMV) was conserved before and after normalization. We performed modulation for only the non-linear portion of the transformation. This procedure results in gray matter maps that are corrected for overall brain size, but reflect local volumetric changes due to non-linear spatial normalization. Non-modulated normalized gray matter density (GMD) images preserve the original MR signal intensity of each voxel, which can allow detection of relatively subtle changes in tissue contrast, as opposed to changes in volume, which can be more sensitively detected in the analysis of modulated images (GMV). Thus, the density refers to voxel intensities obtained after spatial normalization without performing the modulation step.

Non-modulated GMD maps were smoothed with an 11 mm full-width at half-maximum (FWHM) Gaussian kernel (i.e., GMD Images), while a 10mm FWHM smoothing kernel was applied to the modulated images (i.e., GMV Images). Since the modulation process itself imparts some smoothness to the images, the applied smoothing kernels were chosen such

that the final smoothness of the non-modulated and modulated images would be roughly equivalent (measured smoothness of the final GMD and GMV images was 14.3 × 15.8 × 14.3 mm and 14.0 × 15.5 × 14.0 mm, respectively). An identical procedure was applied to the preprocessing of white matter images; to reduce partial volume effects and exclude results at the border between GM and WM, we applied an absolute threshold mask=0.25. Both WM density (WMD) and volume (WMV) were compared across groups.

2.4 Statistical analyses

To examine demographic and clinical differences between groups, one-way ANOVAs were used for continuous measures and chi-square tests were used for categorical measures, where the significance threshold was set at $p<0.05$, two-tailed.

Voxel-wise analyses were performed within the framework of the general linear model. Regional differences in GMD and GMV between groups were investigated using analysis of covariance with age and gender as covariates-of-no-interest and group as the main factor. At the voxel level, the exploratory full-brain statistics were performed with a statistical threshold set to $p_{uncorrected}<0.001$. At the cluster level, due to the non-uniform smoothness of the VBM data (Ashburner and Friston, 2000), we used a non-stationary random-field theory cluster size test (Hayasaka et al., 2004) with a cluster size threshold set at $p<0.05$, Family-Wise Error (FWE) corrected for multiple comparisons. The coordinates localizing the peak voxel t-value within each cluster were converted from Montreal Neurological Institute (MNI) spatial array to the stereotaxic array of Talairach and Tournoux (1988) using a nonlinear transformation (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach). To balance Type I and II error, however, clusters corresponding to $0.05<p_{corrected}<0.1$ also were reported. The same statistical approach was implemented for regional WMD and WMV analyses.

Pearson correlations were used to explore the association between the proportion of GMV and GMD in the peak loci from the significant clusters from the main group analyses and the following clinical variables: time spent unmedicated, time spent in remission, total duration of illness, depression severity assessed using the Montgomery-Åsberg Depression Rating Scale (MADRS) (Montgomery and Åsberg, 1979). Percentage of gray matter was extracted using the “plot function” implemented in SPM5. As a complete medical record was not available for many of our subjects, we were unable to sum with sufficient accuracy, the total duration of past antidepressant drug treatment or the total number of depressive episodes. Therefore, we indirectly addressed the effect of past drug treatment and cumulative illness on brain morphometry using historical information regarding the length of time elapsed since most recent antidepressant drug exposure and the total duration of illness from the onset of depression, respectively.

These secondary analyses were performed separately for each group to avoid potential interactions between the group status and the clinical variables. Given the exploratory nature of those post-hoc analyses, results are reported without correcting for the number of comparisons performed.

3. Results

3.1 Demographic data

Demographic and clinical characteristics of the participants appear in Table 1. The mean age and gender distribution did not significantly differ across the HC, dMDD and rMDD groups. The dMDD and rMDD groups did not significantly differ on illness duration or proportion of subjects who were drug-naïve, while we observed a significantly longer time without medications for the rMDD compared to the dMDD subjects. While three weeks and three

months were the *minimal* drug-free periods for being included in the study for the dMDD and rMDD groups, respectively, the mean time periods spent unmedicated prior to scanning were substantially longer (table 1). Of the 58 dMDD subjects, 43 (74.1%) either met DSM-IV criteria for chronic illness (duration of current depressive episode >2 years) or manifested an intermittent illness course with a current depressive episode lasting <2 years but without recovery between the preceding episodes; 15 (25.9%) had an episodic course of illness with inter-episode recovery.

3.2 Voxel-wise analysis of between-group gray matter difference

The dMDD group had lower GMD than the HC group in the superior frontal gyrus bilaterally and the left middle frontal gyrus (Table 2, Figure 1a). The dMDD subjects also showed reduced GMV compared to HC subjects in the left inferior frontal gyrus and the right middle and superior frontal gyri (Table 3, Figure 2a).

With respect to GMD, the dMDD subjects showed lower GMD than the rMDD subjects in the left middle frontal gyrus, the right superior temporal gyrus, the cuneus bilaterally, the right superior temporal gyrus and the right superior and inferior temporal gyri (Table 2; Figure 1b). With respect to GMV, the dMDD subjects showed lower GMV than the rMDD subjects in the left superior, middle and inferior frontal gyri, the right superior temporal gyrus, and the pregenual and subgenual anterior cingulate cortex (Table 3; Figure 2b).

In the rMDD group, no region was identified in which the GMD or GMV was reduced compared to the HC group. The rMDD group showed significantly greater GMD than the HC group in the dorsal striatum bilaterally, the superior temporal gyrus bilaterally, the right postcentral gyrus and the right inferior frontal gyrus (Table 2, Figure 1c). The rMDD group also showed greater GMV than the HC group in the pregenual ACC and the right ACC (Table 3, Figure 2c).

3.3 Exploratory analyses of the effects of clinical variables on gray matter density and volume

To explore the effects of clinical heterogeneity of the sample in terms of including drug-naïve along with previously medicated subjects, we investigated group differences in GMD or GMV values extracted from the peak loci where significant differences were identified in the entire sample (tables 2 and 3). When the drug-naïve subjects were excluded from analysis the results remained significant in all the brain regions reported.

To evaluate the relationship between gray matter and illness course in the dMDD group, we compared the proportion of gray matter between dMDD patients with episodic course of illness and dMDD patients with a chronic/intermittent course. We found that patients with an episodic course did not differ from patients with a chronic/intermittent course of illness in terms of GMV or GMD, with the exception of the left precuneus where patients with an episodic course of illness showed lower GMD than subjects with a chronic/intermittent course of illness ($t = -2.02$; $p<0.05$).

The exploratory correlational analyses between gray matter and time spent unmedicated or in remission were performed using GMD and GMV values extracted for each subject from the loci where significant effects of diagnosis were detected (tables 2 and 3, respectively). In the dMDD group the time spent unmedicated was positively correlated with GMD in the left middle frontal gyrus ($r = 0.30$; $p< 0.05$; $x= -27$, $y= 28$, $z= 42$) and the precuneus ($r = 0.36$; $p< 0.05$; $x= -13$, $y= -42$, $z= 49$) and was negatively correlated with GMD in the inferior parietal lobule ($r = -0.31$; $p < 0.05$; $x= 40$, $y= -45$, $z= 49$). No regions were identified where time unmedicated correlated with GMV in the dMDD group at $p_{uncorrected}<0.05$. In the rMDD group no correlation between time unmedicated and either GMD or GMV

reached $p_{uncorrected}<0.05$, although trends were observed towards a positive correlation between GMD and time spent in remission in the precuneus bilaterally (r = 0.38; $p < 0.06$; x= −13, y= −42, z= 49 and r = 0.38; $p < 0.06$; x= 14, y= −81, z= 42).

In the rMDD group illness duration was inversely correlated with GMD in the cuneus (r = −0.69; $p < 0.001$; x= −3, y= −91, z= 12) and the putamen/insula bilaterally (r = −0.49; $p< 0.01$; x= −33, y= −5, z= −5 and r = −0.51; $p< 0.01$; x= 36, y= −11, z= −5, respectively), and with the GMV in the left superior frontal gyrus (r = −0.43; $p < 0.05$; x= −28, y= 50, z= 22). No regions were identified where illness duration correlated with GMV or GMD in the dMDD group at $p_{uncorrected}<0.05$.

Finally, to assess the impact of disease severity on gray matter controlling for time unmedicated, we performed partial correlation analyses between MADRS scores and gray matter volume and density in the dMDD group. The MADRS score trended toward correlating with GMD in the right superior frontal gyrus (r = 0.30; $p = 0.05$; x= 12, y= 49, z= 41) and with GMV in the superior frontal gyrus (r = 0.26; $p < 0.10$; x= −28, y= 50, z= 22).

3.4 Voxel-wise analysis of between-group differences in white matter

The comparison of WMD and WMV across groups appears in Supplementary Figure 1. No difference in WMD was identified which remained significant after correcting for multiple comparisons. A trend toward decreased WMD in the rMDD subjects versus the HC subjects was observed in the left ventrolateral prefrontal cortex (x= −29, y= 32, z= 9; k= 1079; *p(corrected)*< 0.1; Suppl. Figure 1a). One group difference in WMV remained significant after correction for multiple comparisons: the dMDD subjects showed decreased WMV than the rMDD subjects in the white matter situated between the dorsal striatum and the middle frontal gyrus (x= 24, y= 8, z= 29; k = 3293; *p(corrected)* <0.005; Suppl. Figure 1b).

4. Discussion

This study constitutes the largest morphometric study of *unmedicated* MDD subjects and the first to include a sample of *unmedicated* MDD subjects in full remission. The currently depressed subjects showed reduced prefrontal cortical GM volume and density as compared to both HC subjects and remitted MDD subjects. The areas of decreased GM in dMDD patients versus healthy controls (tables 2 and 3) localize to the areas of the superior, middle and inferior frontal gyri corresponding to the dorsomedial (DMPFC), dorsal anterolateral (DALPFC), and ventrolateral prefrontal cortices (VLPFC), respectively. The GM also was reduced in currently depressed versus currently remitted MDD subjects in the superior, middle and inferior frontal gyri on the left side, the left insula, the precuneus bilaterally, the right inferior and superior parietal lobule, the right superior temporal gyrus, the pregenual ACC and the left subgenual ACC. Notably, several of the PFC regions (frontal polar cortex, DMPFC, VLPFC, pre- and subgenual ACC) where we detected GM differences in currently depressed MDD subjects compared to HC and/or remitted MDD subjects form part of the “visceromotor network”, which plays major roles in modulating autonomic and behavioral responses to emotional stimuli (Price and Drevets, 2009). Evidence from PET-glucose metabolism studies suggests that this network is characterized by elevated metabolism in the depressed phase relative to the remitted phase of MDD, putatively reflecting heightened glutamatergic transmission during depression (reviewed in Price and Drevets, 2009). Elevated glutamatergic transmission in depression conceivably may lead to excitoxic damage and cellular loss, dendritic reshaping and consequent gray matter morphometric abnormalities, consistent with the histopathological changes identified in post mortem studies of MDD (reviewed in Price and Drevets, 2009).

The GM reduction in the DALPFC and DMPFC identified herein in *unmedicated* depressed MDD subjects versus HC and remitted MDD subjects is consistent with the findings from previous VBM studies of *medicated* depressed MDD subjects (Vasic et al., 2008; Leung et al., 2009; Frodl et al., 2008a; Frodl et al., 2010). Notably, *post mortem* studies of MDD found reduced glial cell number and density and reduced GABAergic interneuron density in these regions (Rajkowska et al., 1999, 2001, 2007). Gray matter abnormalities in the DALPFC and DMPFC might hold prognostic significance, as Li et al. (2010) found reduced GM in the DALPFC in MDD subjects who proved nonresponsive to subsequent treatment with antidepressant drugs, but not in MDD subjects who remitted after subsequent antidepressant pharmacotherapy. The DALPFC and DMPFC gray matter reductions we observed in depressed MDD subjects relative to remitted MDD subjects potentially extends the observation of Li et al. to *unmedicated* MDD patients who experience prolonged remission after a depressive episode, supporting the hypothesis that the absence of DALPFC abnormalities is associated with better long-term outcome.

The unmedicated MDD subjects *in full remission* showed no areas of significantly reduced GM compared to the HC subjects. This negative finding, taken together with the findings that dMDD subjects show DMPFC and DALPFC abnormalities compared to both HC and rMDD subjects, suggests that either these abnormalities reverse during prolonged symptom remission, or that they exist in MDD subjects who are predisposed to develop chronic or intermitent illness, but not in MDD subjects who have the capacity to remain in remission while unmedicated. Compatible with this latter possibility, three-fourths of the dMDD subjects displayed a chronic/intermittent illness course, whereas the rMDD subjects by definition displayed an episodic illness course with full inter-episode recovery. The chronic/intermittent illness course generally represents a more severe and debilitating subphenotype than that associated with an episodic course with full and prolonged recovery between episodes (Angst et al., 2008). Thus, the differential effects of clinical remission versus persistent depressive symptoms on GM in these regions conceivably may reflect a sampling bias that yielded distinct illness subphenotypes. The present findings add to the evidence from functional imaging studies that depressed patients with a more severe course of illness are characterized by more extensive frontolimbic abnormalities as compared to subjects who display a more benign course (Lui et al., 2009; Wu et al., 2010; Zhang et al., 2009).

Furthermore, we unexpectedly detected several regions where rMDD subjects showed *greater* GM than dMDD or HC subjects, including the pregenual and subgenual ACC, the left frontal polar cortex, the right inferior parietal lobule and the superior temporal gyrus (Tables 2,3; figure 2). Since the remitted MDD subjects were studied cross-sectionally, we could not determine whether these areas of increased GM arose during effective treatment or sustained remission as adaptive compensatory changes, or whether they instead represented trait-like, developmental differences that predated illness-onset. In either case such changes conceivably might have conferred resilience against the development of chronic or recurrent depression.

Another possibility is that the areas of increased GM reflect neurotrophic effects of past antidepressant drug exposure (Duman and Monteggia, 2006). Our data argue against the possibility that simple exposure to past antidepressant pharmacotherapy explains the increased GM in the rMDD subjects, since the proportions of dMDD subjects and rMDD subjects who previously were exposed to antidepressant drugs were similar (neither the proportion of subjects who were drug naïve nor the mean illness duration differed significantly between these groups). Our data further argue against the possibility of an effect of antidepressant drugs on GM volume that rapidly subsides after treatment discontinuation, since 22 of the 27 rMDD subjects had been unmedicated for more than one year. Similarly, while we observed an inverse correlation between the length of time since

last psychotropic drug exposure and GMD in the inferior parietal cortex in the *depressed* MDD subjects, there were correlations in the opposite direction in the middle frontal gyrus and precuneus, and these associations did not approach significance in the *remitted* MDD subjects. The lack of a significant relationship between time unmedicated and GMD in the rMDD subjects suggests that the increased GM in the rMDD subjects relative to both the HC and dMDD subjects was unlikely to have been driven by effects of past antidepressant pharmacotherapy. Nevertheless, in the absence of longitudinal studies which control for medication effects on GM, we cannot rule out the possibility that past antidepressant pharmacotherapy exerted neurotrophic effects that were unique to the subgroup who subsequently was able to remain in remission despite treatment discontinuation. Indeed, there is accumulating evidence from cross-sectional (Caetano et al., 2006; Yucel et al., 2009) and longitudinal studies (Frodl et al., 2008b; Chen et al., 2007; Costafreda et al., 2009) that GM morphometry might provide biomarkers associated with antidepressant response (Yucel et al., 2009; Chen et al., 2007) and prolonged remission (Frodl et al., 2008b). Thus, given the cross-sectional nature of the present investigation, the neurobiological mechanisms underlying the increased GM in the rMDD remain unclear and warrant further investigation in studies with a longitudinal design.

We also found evidence of decreased white matter in patients with dMDD and rMDD. White matter abnormalities were localized to the WM of the left VLPFC in the rMDD group, and in the WM dorsal to the striatum in the dMDD group (Suppl. Figure) Notably, the left VLPFC (part of left inferior frontal gyrus) has been shown to contain abnormally reduced GM in depressed MDD subjects both herein (table 3) and in previous *post mortem* studies (Bowen et al. 1989). These findings of WM pathology in MDD are consistent with emerging evidence of white matter pathology in depression detected using VBM (Frodl et al., 2010) and other MRI techniques (e.g., diffusion tensor imaging) (Kieseppä et al., 2010), and with evidence that oligodendroglial cell counts and myelin basic protein concentrations are reduced in the PFC in MDD (reviewed in Price and Drevets, 2010).

A limitation to the generalizability of our results was that patients who had a single, non-chronic MDE were excluded from participation. For example, the rMDD subjects were required to manifest at least 2 MDE before achieving remission. It is conceivable that differences in subject selection may account for differences in the results across VBM studies. For example, Zou and colleagues described reduced hippocampal volumes in first-episode, treatment-naive subjects with MDD, which were not represented within our study sample (but see below). In contrast, most of our rMDD and dMDD subjects manifested a relatively long illness duration, and less than one-fourth of each sample was drug-naïve (table 1). Finally, the participants enrolled in this study were mostly outpatients; therefore, it remains unclear whether our findings are generalizable to inpatients with more severe depression.

Some limitations of the VBM method also merit comment and may contribute to our negative findings in the dMDD subjects in subcortical structures, such as the hippocampus or amygdala. Nevertheless, while some previous VBM studies detected reductions in these structures in MDD (Tang et al., 2007; Wagner et al., 2008; Vasic et al., 2008), the sensitivity of the VBM procedure for detecting changes in subcortical structures, such as the hippocampus, is markedly lower than that of manual segmentation or of some recently developed semi-automated segmentation algorithms (Bergouigan et al., 2009). Notably, Frodl and colleagues (Frodl et al., 2008a), who conducted the largest VBM study and the only study to use correction for multiple comparisons across the whole brain, did not find volumetric differences between MDD patients and healthy controls in the amygdala or hippocampus. Likewise, we also did not find evidence of structural abnormalities in rMDD subjects, although hippocampal volumetric abnormalities have been documented in remitted

MDD subjects by our laboratory using manual segmentation of high-resolution MRI (Neumeister et al., 2005). These conflicting results may be attributable to limitations inherent within the VBM method: First, during segmentation of some subcortical structures the boundaries between gray and white matter can be detected more accurately by the human eye than by automated computer algorithms. Second, the VBM approach involves vastly more independent statistical comparisons than manual segmentation, so the threshold for statistical significance becomes much higher for VBM after adjusting for multiple testing. This limitation in statistical sensitivity is particularly problematical since the effect size of volumetric abnormalities in subcortical structures in MDD is relatively small. The chief advantages of the VBM technique thus remain its capability for detecting inherent differences between groups in cortical regions that cannot be reliably delimited using manual segmentation or where too little knowledge exists to guide region-of-interest definition *a priori*. A final limitation of VBM is with the interpretability of the results in regards to actual physical properties of the tissue. In particular, the gray matter density measure cannot be literally interpreted as neuronal density. Although influenced by the level of contrast in the image, the gray matter density can be interpreted as a reflection of the proportion of gray matter within a given voxel.

4.1 Conclusions

In conclusion, in a relatively large sample of unmedicated subjects with MDD, we found neuromorphometric abnormalities in the prefrontal cortex, the anterior cingulate cortex, the insula and other regions belonging to the visceromotor network. Unmedicated MDD subjects in full remission did not show similar evidence of reduced GM, but instead demonstrated areas of increased GM relative both to healthy and depressed controls. Our study design was cross-sectional, so it remains unclear whether the structural abnormalities found in each MDD subgroups arose as a consequence of prolonged remission versus persistent depression, or instead predated illness-onset as an underlying susceptibility to more versus less severe MDD illness courses. Future studies employing longitudinal designs that track subjects from high-risk populations through the transition into affective illness are needed to investigate the relationships between neuromorphometry, clinical course and antidepressant treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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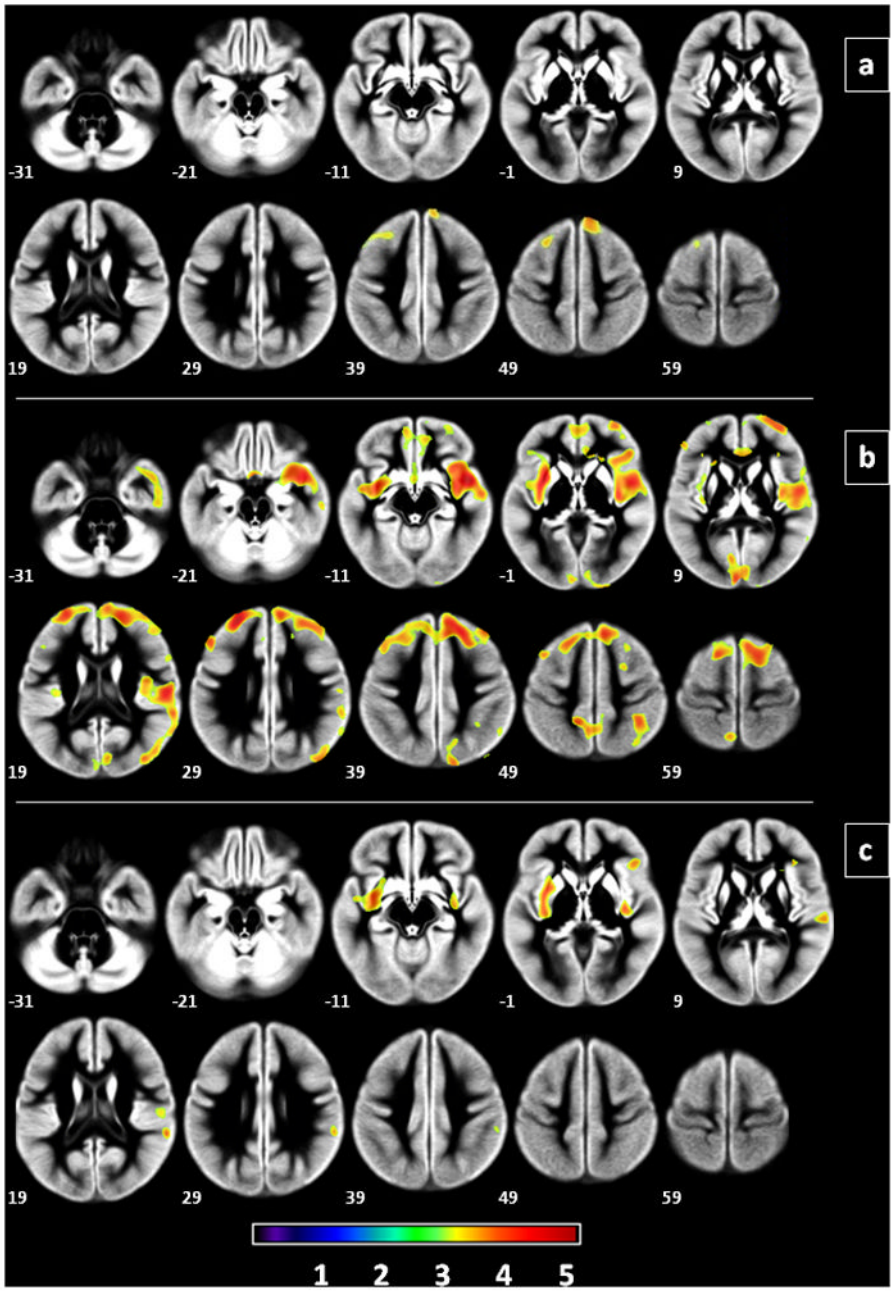

Figure 1.

Regions where gray matter *density* was reduced in currently depressed (N=58) subjects with major depressive disorder compared to healthy controls (N=107) (**a**) and to currently remitted MDD subjects (N=27) (**b**). In this figure are also displayed regions were currently remitted MDD subjects showed greater gray matter density than healthy controls (**c**). Results are superimposed on axial slices of the average GM map of the 192 subjects participating in this study. The axial slices shown parallel the axial plane containing both the anterior and posterior commissures (defined as z=0) and extend from 31 mm below to 59 mm above this plane (i.e., z = −31 to 59 mm). Images are displayed at $p<0.10$ FWE cluster-corrected statistics using the MRIcron software (http://www.sph.sc.edu/comd/rorden/mricron/).

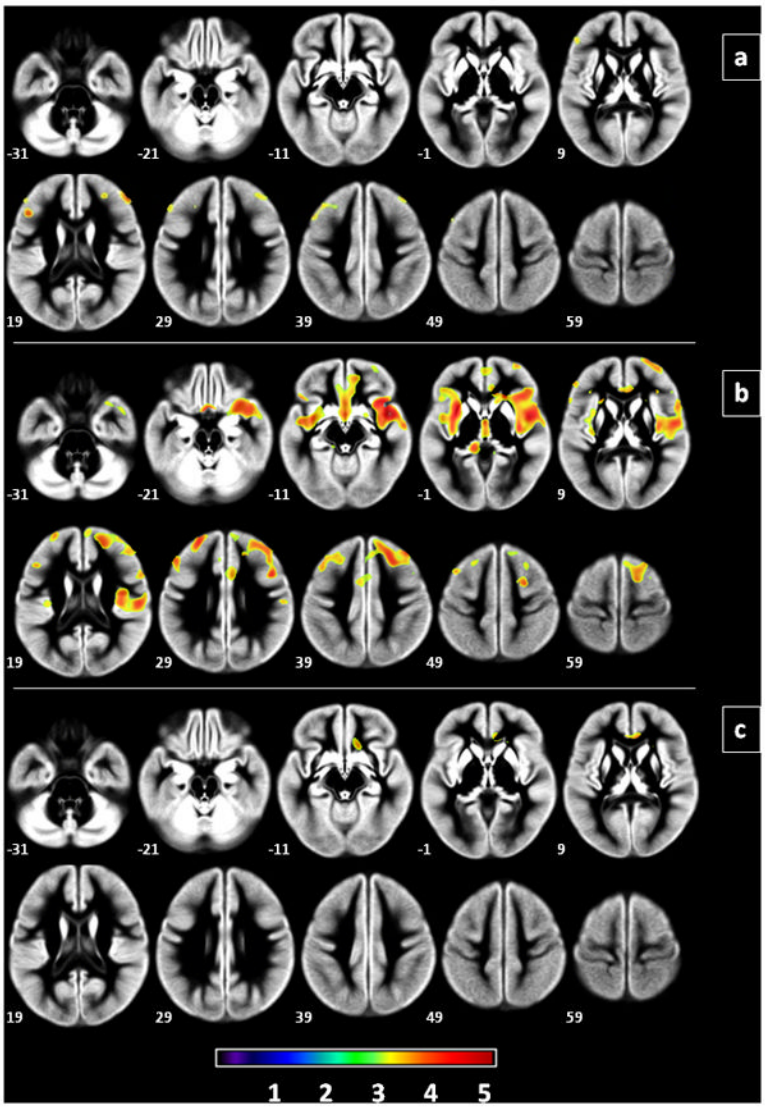

Figure 2.

Regions where gray matter *volume* was reduced in currently depressed subjects with major depressive disorder (N=58) compared to healthy controls (N=107) (**a**) and to currently remitted MDD subjects (N=27) (**b**). In this figure are also displayed regions where currently remitted MDD subjects showed greater gray matter volume than healthy controls (**c**). Results are superimposed on axial slices of the average GM map of 192 subjects participating in this study (z = −31 to 59 mm). Images are displayed at p<0.10 FWE cluster-corrected statistics using MRIcron software (http://www.sph.sc.edu/comd/rorden/mricron/).

Table 1

Demographic and clinical characteristics of the study samples*

Variable	Healthy Controls (n=107)	dMDD (n=58)	rMDD (n=27)	*P*
Age, y	36.2 (10.3)	38.8 (11.1)	40.2 (12.2)	0.13
Range	*19–60*	*20–60*	*18–61*	
Gender (F:M)	60:47	37:21	21:6	0.11
MADRS	…	26 (7.6)	1.6 (2.3)	<0.001
Range		*10–42*	*0–9*	
Duration of illness, y	…	18.4 (10.5)	15.1 (12.2)	0.20
Range		*2–40*	*1–37*	
Drug naïve (%)	…	14 (24.1)	6 (22.2)	0.85
Time unmedicated, m	…	21.1 (31.1)	53.9 (60.9)	0.005
Range		*1–130*	*3–240*	
Episode duration, m,	…	47.7 (74.1)	…	…
Range		*1–384*		
Time remitted, m,	…	…	39.6 (41.7)	…
Range			*3–156*	

Abbreviations: dMDD, currently depressed subjects; rMDD, currently remitted subjects; MADRS, Montgomery and Åsberg Depression Rating Scale; m, months; y, years.

*Continous variables are reported as Mean (Standard Deviation)

Table 2

Regional differences in mean gray matter *density* between the currently depressed subjects with major depression (n=58) compared to healthy controls (n=107) and currently remitted subjects (n=27)

Region	*k*	FWE	T	Local Maximum		
HC > dMDD						
Right Superior Frontal G	2157	0.071	4.04	12	49	41
Right Superior Frontal G			3.77	11	56	36
Right Superior Frontal G			3.17	22	35	46
Left Middle Frontal G	2736	0.038	3.72	−27	28	42
Left Superior Frontal G			3.60	−32	36	30
Left Superior Frontal G			3.45	−21	32	47
rMDD > dMDD						
Left Frontal Polar Cortex	95942	<0.001	5.31	−28	50	22
Right Superior Temporal G			5.22	50	3	−7
Left Insula			5.19	−33	0	−3
Left Precuneus	2974	0.03	4.32	−13	−42	49
Precuneus			3.88	−3	−50	47
Left Precuneus			3.77	−6	−56	55
Cuneus	6240	<0.001	4.27	−3	−91	12
Left Cuneus			4.05	−7	−71	10
Right Cuneus			3.90	8	−85	8
Right Inferior Parietal Lobule	4213	0.009	4.19	40	−45	49
Right Inferior Parietal Lobule			3.68	51	−52	41
Right Superior Parietal Lobule			3.65	36	−57	51
Right Precuneus	1912	0.094	4.04	14	−81	42
Right Precuneus			3.45	11	−68	36
Right Precuneus			3.43	16	−62	40
rMDD > HC						
Left Putamen/Insula	6705	0.001	4.70	−33	−5	−5
Left Putamen/Insula			4.61	−34	−14	−4
Left Superior Temporal G			3.50	−48	−4	−11
Right Putamen/Insula	1993	0.085	4.49	36	−11	−5

Region	*k*	FWE	T	Local Maximum		
Right Inferior Frontal G	2164	0.070	4.15	40	28	1
Right Putamen/Insula			3.55	24	24	4
Right Inferior Parietal Lobule	3237	0.023	4.14	61	−36	23
Right Superior Temporal G			4.14	58	−23	12
Right Postcentral G			3.61	58	−6	14

Abbreviations: G, gyrus; k, number of voxels in the cluster; HC, healthy control subjects; dMDD, currently depressed subjects; rMDD, currently remitted subjects; FWE, familywise error corrected at the cluster level; x, y, z show coordinates in mm from the sterotaxic origin, with positive y denoting anterior, positive z superior, and positive x to the right of midline (Talairach and Tournoux, 1988); T, T-score

Table 3

Regional differences in mean gray matter *volume* between the currently depressed subjects with major depressive disorder (n=58) compared to healthy controls (n=107) and currently remitted MDD subjects (n=27)

Region	*k*	FWE	T	Local Maximum		
HC > dMDD						
Left Inferior Frontal G	3330	0.017	4.24	−47	24	17
Left Inferior Frontal G			3.74	−49	38	10
Right Superior Frontal G			3.59	−37	36	30
Right Middle Frontal G	1799	0.091	3.97	51	41	16
Right Superior Frontal G			3.94	40	41	30
Right Superior Frontal G			3.55	29	44	16
rMDD > dMDD						
Right Superior Temporal G	81120	<0.001	5.56	49	4	−7
Subgenual Anterior Cingulate G			5.00	−2	9	−16
(Infralimbic Cortex)						
Right Subgenual Anterior Cingulate G			4.89	13	25	−7
Left Superior Frontal G	7667	<0.001	4.56	−28	50	22
Left Middle Frontal G			4.21	−42	20	40
Left Inferior Frontal G			4.20	−48	24	20
rMDD > HC						
Right Subgenual Anterior Cingulate G	2982	0.024	4.95	13	24	−8
Right Pregenual Anterior Cingulate G			4.06	7	31	5
Pregenual Anterior Cingulate G			3.54	−2	31	5

Abbreviations: G, gyrus; k, number of voxels in the cluster; HC, healthy control subjects; dMDD, currently depressed subjects; rMDD, currently remitted subjects; FWE, familywise error corrected at the cluster level; x, y, z show coordinates in mm from the sterotaxic origin, with positive y denoting anterior, positive z superior, and positive x to the right of midline (Talairach and Tournoux, 1988); T, T-score

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